

**FERMENTATION AND PURIFICATION OF LIPASE
BY *BURKHOLDERIA METALLICA*
USING COLUMN REACTOR**

NUR EZZREEN ZAFHRINA BINTI MOHAMAD REDZA

UNIVERSITI SAINS MALAYSIA

JUNE 2020



**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERTAAN DISERTAI MUTAKHIR
SATU (1) NASKAH**

Nama Penyelia: DR TAN JOO SHUN

Bahagian: TEKNOLOGI BIOPROSES

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh
Encik/Puan/Cik NUR EZZREEN ZAFHRINA BINTI MOHAMAD REDZA

mengemui disertainya sebagaimana yang dipersetujui oleh Panel Pemeriksa di Viva
Vocanya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembetulan/pindaan
yang dilaksanakan oleh calon.

Sekian, terima kasih.



DR. TAN JOO SHUN
(Tandatangan dan cap)
Pusat Pengajian Teknologi Industri
Universiti Sains Malaysia
11800 Pulau Pinang, Malaysia
Tel: 04-653 6376

16/7/2020

Tarikh



**FERMENTATION AND PURIFICATION OF LIPASE
BY *BURKHOLDERIA METALLICA*
USING COLUMN REACTOR**

by

NUR EZZREEN ZAFHRINA BINTI MOHAMAD REDZA

A dissertation submitted in the partial fulfillment of the requirements for the degree of
Bachelor of Technology (B.Tech) in the field of Bioprocess Technology
School of Industrial Technology
Universiti Sains Malaysia

June 2020

DECLARATION BY AUTHOR

This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.



.....

Nur Ezzreen Zafhrina Binti Mohamad Redza

Date: June 2020

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my advisor Dr Tan Joo Shun for the continuous support of my bachelor study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my study. Besides my advisor, I would like to thank my laboratory assistant Puan Najma for her insightful comments and encouragement, but also for the hard question which incited me to widen my research from various perspectives.

I thank my fellow friends which is Nur Syuhada Binti Baharuddin for giving me lot of moral support and not to forget Nur Elleena Zaffira binti Mohamad Redza for having time to give a good advice on my thesis writing. Last but not the least, I would like to thank my parents and to my brothers and sister for supporting me spiritually throughout writing this thesis and my life in general.

My fellow bachelor students, especially my classmates should also be recognized for their support. My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Their views and tips are very useful indeed.

Nur Ezzreen Zafhrina Binti Mohamad Redza

June 2020

TABLE OF CONTENTS

	PAGE
Declaration by author	ii
Acknowledgement	iii
Table of contents	iv
List of tables	vii
List of figures	viii
List of abbreviations and symbols	ix
Abstrak	xi
Abstract	xiii
 CHAPTER 1: INTRODUCTION	
1.1 Research background	1
1.2 Problem statement	4
1.3 Research scope and objective	5
 CHAPTER 2: LITERATURE REVIEW	
2.1 Bacteria Strain: <i>Burkholderia metallica</i>	6
2.2 Lipase	7
2.2.1 Sources of Lipase	7
2.2.2 Application of microbial lipase	8
	iv

2.3	Plant lipase purification and recombinant production – challenges and scope	11
2.4	Separation, purification and downstream recoveries approach for lipase enzyme	12
2.4.1	Aqueous Impregnated Resin System (AIRS)	
2.4.1 a	Principle	13
2.4.1 b	Protocol	14

CHAPTER 3: METHODOLOGY

3.1	Design of study	15
3.2	Methods	
3.2.1	Experiment Flow Chart	16
3.2.2	Microorganism and Inoculum preparation	17
3.2.3	Preparation of fermentation feedstock	18
3.2.4	Determination of lipase activity and total protein content	18
3.3	Investigation on effects of various parameters on PF and yield (%)	
3.3.1	Type of salt	19
3.3.2	Purification stage (Setup AIRS)	19
3.3.3	Study the effect of purification parameters	20
3.4	Optimization of the extraction procedure employing AIR systems	21
3.5	Determination of specific activity, yield and purification factor	21

CHAPTER 4: RESULT AND DISCUSSION

4.1	Purification process on various parameters	23
4.1.1	Effect of type and concentration of salt on enzyme activity	24
4.1.2	Composition of PEG- Sodium Citrate (PEG-salt composition)	25
a.	Influence of addition of NaCL	28
b.	Hydrophobicity	30
c.	Molecular weight (MW) and PEG concentration	31
4.1.3	Effect of pH value on PF and yield (%)	32
4.1.4	Effect of flowrate (ml/s) toward PF and yield (%)	34
4.1.5	Effect of extraction time on PF and yield (%)	35
4.2	Comparison of lipase production by <i>B.metallica</i> and previously reported study utilizing other bacteria types	36

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1	Conclusion	37
5.2	Recommendation	38
	References	40
	Appendices	46

LIST OF TABLES

Table	Caption	Page
2.1	Plant lipases with their corresponding plant parts used for extraction	11
4.1	Effect of composition of PEG and salts on PF and yield (%) of purified lipase	26
4.2	Critical properties used to achieve and factor affecting phase separation of particular protein/enzyme	29

LIST OF FIGURES

Figure	Caption	Page
2.1	Schematic diagram of AIRS	14
3.1	Flowchart of the methodologies carried out in this study	16
4.1	Effect of type of salt on lipase activity	24
4.2	Effect of pH value on PF and yield (%) of purified lipase	32
4.3	Effect of flowrate (ml/s) on PF and yield (%) of purified lipase	34
4.4	Effect of extraction time on PF and yield (%) of purified lipase	35

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Caption
ATPS	Aqueous Two Phase System
ATPE	Aqueous Two Phase Extraction
AIRS	Aqueous Impregnated Resin System
TAPPIR	Tunable Aqueous Polymer-Phase Impregnated Resin
M _w	Molecular Weight
ml	Milliliter
mm	Milimeter
min	Minute
Nm	Nanometer
s	Second
Sp	Species
R-P HPLC	Reverse-Phase High Performance Liquid Chromatography
P _f	Purification Factor
PEG	Polyethylene Glycol
pI	Isoelectric Point
pH	Potential of hydrogen
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

μmol Micromole

μl Microliter

Symbol

Caption

$^{\circ}\text{C}$ Degree of celcius

- Minus

\div Divide

= Equal to

> Greater than

< Less than

% Percentage

**FERMENTASI DAN PEMURNIAN LIPASE OLEH BURKHOLDERIA METALLICA
MENGUNAKAN CAIRAN REAKTOR COLUMN YANG DIRESAPI
YANG DITETAPKAN**

ABSTRAK

Dalam hasil kerja ini, fermentasi dilakukan untuk bahan makanan dan pemurnian lipase oleh *Burkholderia metallica* menggunakan reaktor lajur. Parameter yang disasarkan untuk penyelidikan ini seperti kestabilan garam, jenis dan kepekatan Polietilena glikol (PEG) dan garam, pH larutan pengestrakan dan kadar aliran ml/s untuk melihat prestasi sistem pemurnian ekstraktif. Sistem resin berair impregnated (AIRS) digunakan menggunakan manik-manik kaca berliang yang dicangkokkan dengan polietilena glikol (PEG). Kajian terhadap AIRS dilakukan dengan pelbagai berat molekul polietilena glikol (PEG) (PEG 2,000, PEG 4,000, PEG 6,000) dan pelbagai jenis garam (natrium sitrat, kalium sitrat dan natrium asetat) sebagai komponen fasa. Pengekstrak cecair PEG tidak digerakkan pada penyokong lengai (manik kaca berliang) dan sesuai dengan keadaan fasa berair untuk mengurangkan masalah pengestrakan dua fasa berair (ATPE). Teknik ini menggabungkan pengestrakan cecair-cecair dengan mudah dengan operasi kromatografi lajur. Kemudian sasarkan biomolekul yang diserap pada keadaan berair (fasa pegun) dan kotoran dikeluarkan dari aliran. Hasil daripada eksperimen One-factor-at-a-time (OFAT) menunjukkan bahawa komposisi lipase optimum 20% (w/w) PEG 6,000 resin yang diresapi dengan larutan pengestrakan 5% natrium sitrat pada pH 7,7, 4% natrium klorida (NaCl) dan 20% (w/w) tetap dari pemuatan kasar pemisahan lipase yang lebih baik. Kesimpulannya hasil kajian menunjukkan kaedah satu kali pemurnian, kaedah pemisahan secara tidak membahayakan tubuh badan manusia dan ringan, langkah menghilangkan pemisahan fasadan juga pengurangan jejak ATPE yang menghasilkan faktor pemurnian peningkatan tinggi AIRS

yang merupakan Resin yang Diterapkan Fasa Polimer Berair yang ditingkatkan (TAPPIR), digunakan untuk pemurnian lipase dari *Burkholderia metallica*.

**FERMENTATION AND PURIFICATION OF LIPASE BY
BURKHOLDERIA METALLICA USING AQUEOUS
IMPREGNATED COLUMN REACTOR**

ABSTRACT

In this work, fermentation was conducted for the feedstock and purification of lipase by *Burkholderia metallica* using a column reactor. The targeted parameter for this research such as salt stability, types and concentrations of polyethylene glycol(PEG) and salt, pH of extraction solution and flowrate ml/s to see the performance of extractive purification system. Aqueous impregnated resin system(AIRS) is applied using porous glass beads grafted with polyethylene glycol (PEG). An evaluation studies on AIRS were performed with various molecular weight of polyethylene glycol (PEG) (PEG 2,000, PEG 4,000, PEG 6,000) and different type of salts (sodium citrate, potassium citrate and sodium acetate) as phase component. Liquid extractant of PEG was immobilized on the inert support (porous glass beads) and correspond to the aqueous state phase to surmount the drawback of aqueous two phase extraction (ATPE). The technique combines liquid-liquid extraction with ease if operation of column chromatography. Then target biomolecules absorbed on aqueous (stationary phase) and impurities removed from the flow through. One-factor-at-a-time (OFAT) experimental result showed that the optimum composition of lipase purification was 20% (w/w) PEG 6,000 impregnated resin with extraction solution of 5% sodium citrate at pH 7.7, 4% sodium chloride (NaCl) and 20% (w/w) fixed of crude loading have a better separation of lipase. In conclusion the outcome of the studied showed single step purification, biocompatible and mild separation condition, eliminated phase separation step and thus reduced ATPE footprint that results the purification factor of high value reveals AIRS which

is the improved Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR), could be successfully used to purify lipase from *B.metallica*.